respectfully request that the Examiner withdrawn the present rejection for indefiniteness in view of Applicants' amendments.

II. Rejections Of Claims 13-14 Under 35 U.S.C. § 102

The Examiner rejects claims 13-14 under 35 U.S.C. § 102(a) as being anticipated by Burg, et al. or Higuchi, et al. (1993) and under 35 U.S.C. § 102(b) as being anticipated by Higuchi, et al. (1992). In the Office Action, the Examiner indicates that the language regarding the first and second fluorescent signals is directed toward an "intended use" and therefore does not need to be addressed.

Applicants traverse this rejection by amending claim 13 so that the apparatus includes a detection and analysis mechanism which receives the first and second fluorescent signals at a plurality of times, measures the intensities of the first and second fluorescent signals at the plurality of times and produces a plurality of corrected intensity signals, each corrected intensity signal corresponding to a ratio between the intensities of the first and second fluorescent signals at a given time. Support for this amendment appears in the Specification at page 9, lines 11-26.

The specification in claim 13 of a detection and analysis mechanism which includes the function of producing a plurality of corrected intensity signals when the mechanism measures the intensities of the first and second fluorescent signals creates a positive limitation on the operation of the apparatus which must be considered by the Examiner when examining this claim.

Neither Burg, et al. nor the Higuchi, et al. references (Higuchi 1992, Higuchi 1993) teach an apparatus which calculates a ratio between two fluorescent signals and produces a corrected intensity signal in response. Since the references cited do not teach each and every limitation of claim 13, the Examiner's rejection for anticipation of this claim is unsupported and should be withdrawn.

IV. Rejection Of Claims 24-25, 27, 33 and 35 Under 35 U.S.C. § 102

The Examiner rejects claims 24-25, 27, 33 and 35 under 35 U.S.C. § 102(a) as being anticipated by Lee, et al. Claim 24 has been rewritten as new claim 39. Claim 35 has been rewritten in independent form as new claim 42.

Applicants note that Lee, et al. does not teach monitoring the formation of a nucleic acid amplification reaction product in real time. For example, Lee, et al. does not teach the following claim limitations:

> transmitting an excitation beam into the sample holder;
> measuring the intensities of the first and second fluorescent signals before **and** after an amplification of the nucleic acid sequence; or
> calculating corrected intensity signals for before and after an amplification in order to detect the formation of the nucleic acid amplification reaction product based on a change in the corrected intensity signal over time.

Instead, Lee, et al. teaches transferring the reaction mixtures from the PCR tube to a cuvette after the amplification has been completed. See Lee, et al., page 3766, Col. 2. Furthermore, Lee, et al. teaches "if fluorescence can be detected directly in the reaction vessel," affirming the fact that Lee, et al. does not teach how to detect fluorescence directly in a reaction vessel. Rather, detecting fluorescence directly in the reaction vessel is left as a future development.

Since Lee, et al. does not teach the above steps specified in the rejected method claims, Lee, et al. is not an anticipatory reference. Applicants therefore respectfully request that the Examiner withdraw the present rejection.

V. Rejections Of Claims 13-34 and 36 Under 35 U.S.C. § 103

The Examiner rejects claims 13-34 and 36 under 35 U.S.C. § 103 as being unpatentable over the combination of either Burg, et al. or Higuchi, et al. (1993) in view of either Gershoni, et al. or Krause, et al. and Higuchi, et al. (1992) in view of either Gershoni, et al. or Krause, et al.

A. Claim 13 Is Amended To Recite A Positive Limitation Requiring Consideration

With regard to claim 13, the Examiner indicates that the language specifying first and second fluorescent signals is directed toward an "intended use" and therefore does not need to be addressed. Applicants have amended claim 13 to include a detection and analysis mechanism which includes the function of producing a plurality of corrected intensity signals over time when the mechanism measures the intensities of the first and second fluorescent signals. This amendment to the claim creates a positive limitation on the operation of the apparatus which must be considered by the Examiner when examining this claim.

B. Summary Of The Problem Recognized And Solved By This Invention

In the present invention, Applicants recognized that small variations in base line fluorescence due to system based variations exist when monitoring a sample over time. Specification, page 14, lines 4-12, Figure 4 (See variation in peak intensities of TAMRA peaks in Figure 4). Applicants reduce these system based variations by using an internal standard (TAMRA) in combination with a fluorescent indicator for the concentration of the amplification reaction product (FAM). See Figure 4. By calculating the ratio of fluorescence between the fluorescent indicator for the amplification reaction product (FAM) and the internal standard (TAMRA), the RMS of fluctuations in the readout signal is reduced to less than 1% of the average magnitude of the measured ratio. Specification, page 14, lines 8-12, Figure 5.

C. Examiner's Combination Of References Is Improper

In Applicants' previous Amendment mailed March 1, 1996, Applicants objected to the Examiner's combination of Burg, et al. or Higuchi, et al. (1993) or Higuchi, et al. (1992) with Gershoni, et al. or Krause, et al. Applicants renew their objection to this combination of references.

The prior art must teach or suggest making a modification to the prior art in order to render a claimed invention obvious. In re Gordon, 221 USPQ 1125, 1127 (Fed. Cir. 1984). In other words, one most be **motivated** by the prior art to make the modification necessary to arrive at the present invention. In re Vaeck, 947 F.2d 488, 493 (Fed. Cir. 1991). Absent such motivation, a rejection based on a combination of references is unsupported and any rejection based on such a combination must be withdrawn.

Burg, et al. and the Higuchi, et al. references do not teach or suggest the existence of the problem being solved by the present invention, namely, that the base line fluorescence of a **single sample** will vary **over time** due to system based variations. In particular, Applicants note EP 512 334 (Higuchi) which teaches that "the present invention is suitable in conjunction with methods using an internal standard to determine either the relative amount of a target or accurately quantitate the amount of target present prior to amplification." EP 512 334, page 10, lines 36-38. In Example VII, Higuchi teaches the use of an internal standard (positive control DNA) to demonstrate significant increases (greater than 2 standard deviations) in the fluorescence of samples containing a positive control DNA. EP 512 334, page 15, lines 25-40, Figures 4A-B. As can be seen from this teaching, Higuchi was concerned with distinguishing background fluorescence from fluorescence from amplified DNA. However,

Higuchi does not recognize the further problem that the fluorescence measured in a sample can also vary over time due to system based variations. Rather, this type of error is not suggested anywhere but in the present invention.

In the Office Action, the Examiner states that Gershoni, et al. and Krause, et al. "do address the problem of the instant invention: correcting for errors in measurements of fluorescence." Office Action, page 12 (emphasis original). However, neither Gershoni, et al. nor Krause, et al. are concerned with monitoring the progress of an amplification reaction or the correction of system based fluorescence variations in a **single sample over time**. Rather, these references are completely **unrelated to PCR** and involve the **one time quantification** of chlorophyll fluorescence in different samples of photosynthetic cells at **very low temperatures** (77 °K, i.e., -196 °C). These references also use an internal standard in a different manner and for a different purpose than the present invention. Specifically, these references use an internal standard in order to normalize the fluorescence spectra of **different samples** in order to compare the different samples. By contrast, Applicants use an internal standard to reduce the size of a quantification error which is created when the **same sample** is analyzed at **multiple time intervals**.

Based on the disparities between Gershoni, et al. and Krause, et al. and the present invention with regard to the types of assays involved and their operating temperatures, as well as the way in which the internal standard is used, Applicants maintain that Gershoni, et al. and Krause, et al. would not motivate one of ordinary skill to modify Burg or the Higuchi references to further include an internal standard in order to arrive at the present invention. Rather, Applicants are the first to recognize the problem of system based fluorescence variations in a single sample when monitoring the formation of a nucleic acid amplification reaction product in real time and the solution thereto. Accordingly, Applicants are entitled to a patent to their solution to this problem. The Examiner is therefore respectfully requested to withdraw the present rejection for obviousness.

D. Claims Patentable In View Of Unexpected Result

As discussed above in Section B, Applicants recognized that small variations in base line fluorescence due to system based variations exist when monitoring a sample over time. By using an internal standard and calculating the ratio of fluorescence between the fluorescent indicator for the amplification reaction product and the internal standard, Applicants were able to reduce the RMS of fluctuations in the readout signal.

None of the prior art references cited by the Examiner teach or suggest the existence of the problem being solved by the present invention, or its solution. Applicants thus provide a way of improving the accuracy of fluorescence measurements of an amplification reaction being monitored in real time. Since there is no recognition in the art that the accuracy of this type of fluorescence measurements could be improved by eliminating system based errors, Applicants' discovery of this source of error and the claimed way of removing the error provides an unexpected improvement in the operation of devices monitoring amplification reactions in real time, as well the method associated therewith. In view of this unexpected improvement, Applicants maintain that they are entitled to a patent and respectfully request that the Examiner withdraw the present rejection for obviousness.

VI. Rejection Of Claim 37 And 38 Under 35 U.S.C. § 103

The Examiner rejects claim 37 and 38 under 35 U.S.C. § 103 as being unpatentable for obviousness over Burg, et al., Higuchi, et al. (1992) or Higuchi, et al. (1993), in view of either Gershoni, et al. or Krause, et al., and in further view of Renzoni, et al. Claim 38 has been rewritten as new claim 40. Applicants traverse the Examiner's rejection on the same grounds as is specified in Section V of this Amendment.

VII. Rejection Of Claims 26, 28-32, 34 and 36 Under 35 U.S.C. § 103

The Examiner rejects claims 26, 28-32, 34 and 36 under 35 U.S.C. § 103 as being unpatentable for obviousness over Lee, et al. in view of any one of Burg, et al., Higuchi, et al. (1992) or Higuchi, et al. (1993). Applicants traverse the Examiner's rejection on the grounds that none of the cited references teach monitoring the formation of a nucleic acid amplification reaction product in real time. As discussed above, Burg, et al., Higuchi, et al. (1992) and Higuchi, et al. (1993) do not teach or suggest the existence of the problem being solved by the present invention, namely, that the base line fluorescence of a **single sample** will vary **over time** due to system based variations. Meanwhile, Lee, et al. teaches transferring the reaction mixtures from the PCR tube to a cuvette after the amplification has been completed and affirms the fact that detecting fluoresence directly in a reaction vessel is not being taught ("if fluorescence can be detected directly in the reaction vessel"). See Lee, et al., page 3766, Col. 2. These references therefore do not teach

measuring the intensities of a first and second fluorescent signal in a

sample holder before **and** after an amplification of the nucleic acid sequence; and

calculating corrected intensity signals for before and after an amplification in order to detect the formation of the nucleic acid amplification reaction product based on a change in the corrected intensity signal over time.

Since Applicants are the first to recognize the problem of system based fluorescence variations in a single sample when monitoring the formation of a nucleic acid amplification reaction product in real time and the solution thereto, Applicants are entitled to a patent to their solution to this problem. The Examiner is therefore respectfully requested to withdraw the present rejection for obviousness.

VIII. Rejection Of Claims 37-38 Under 35 U.S.C. § 103

The Examiner rejects claim 37-38 under 35 U.S.C. § 103 as being unpatentable for obviousness over Lee, et al. in view of Renzoni, et al. Claim 37 has been canceled and claim 38 has been rewritten as new claim 41. As discussed above in Section IV, Lee, et al. does not teach monitoring the formation of a nucleic acid amplification reaction product in real time. Specifically, Lee, et al. does not teach the following claim limitations:

transmitting an excitation beam into the sample holder;

measuring the intensities of the first and second fluorescent signals before **and** after an amplification of the nucleic acid sequence; or

calculating corrected intensity signals for before and after an amplification in order to detect the formation of the nucleic acid amplification reaction product based on a change in the corrected intensity signal over time.

Instead, Lee, et al. teaches transferring the reaction mixtures from the PCR tube to a cuvette after the amplification has been completed. Since Lee, et al. does not teach the above steps in the method, Applicants submit that the rejected claims are not rendered obvious by the combination of Lee, et al. with Renzoni, et al. and respectfully request that the Examiner withdraw this rejection.

CONCLUSION

In light of the Amendments and the arguments set forth above, Applicants earnestly believe that they are entitled to a letters patent, and respectfully solicit the Examiner to expedite prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is encouraged to telephone the undersigned.

Respectfully submitted,

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